

Docket No: AdVec10IA-C1

Serial No: 09/978,464

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (previously presented) A method for making an infectious adenovirus having enhanced efficiency which comprises contacting a cell with or introducing into a cell:
 - (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are incapable to encode an infectious, replicable or packageable adenovirus; and
 - (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable to encode an infectious, replicable or packageable adenovirus;provided that said first and said second nucleic acid sequences each comprise a head-to-head ITR junction and said first nucleic acid and said second nucleic acid comprise recombinase recognition sites and wherein said first and said second nucleic acids are contacted with a recombinase which recognizes said first nucleic acid and said second nucleic acid recombinase recognition sites; whereby said first and said second nucleic acids recombine to form said infectious adenovirus.
2. (original) The method according to claim 1 wherein said first nucleic acid sequence is a plasmid containing a circularized adenovirus DNA molecule.
3. (previously presented) The method according to claim 2 wherein said plasmid includes a bacterial origin of DNA replication, an antibiotic resistance gene for selection in bacteria,

Docket No: AdVec10LA-C1

Serial No: 09/978,464

1 a deletion or modification in E1 that renders the adenoviral sequences incapable to form
2 infectious virus, or an expression cassette encoding a site-specific recombinase, and
3 combinations thereof.

1 4 (original) The method according to claim 2 wherein said adenovirus DNA has a deletion
2 of an adenoviral packaging signal, or wherein said packaging signal is flanked on either
side by at least one site-specific recombinase recognition site.

1 5 (original) The method according to claim 4 wherein said adenovirus DNA comprises (i) a
2 deletion of, (ii) a modification in, or (iii) sequences flanked with a site-specific
3 recombinase recognition site, of an adenoviral gene selected from the group consisting of
4 adenoviral E1 sequences extending beyond said packaging signal, adenoviral fibre gene
5 sequences, adenoviral E3 gene sequences, adenoviral E4 gene sequences, and
6 combinations thereof.

1 6 (original) The method according to claim 5 wherein said adenovirus DNA has a *lox* site
2 located 5' of a pIX gene.

1 7 (cancelled)

1 8 (original) The method according to claim 1 wherein said second nucleic acid sequence is
2 a plasmid comprising:

- 3 (a) said head-to-head ITR junction, and a packaging signal contained within the
4 leftmost approximately 350 nt of the adenovirus genome;
5 (b) a polycloning site or a foreign DNA or an expression cassette; and optionally,
6 (c) a *lox* P site 3' of said polycloning site, foreign DNA, or expression cassette.

1 9. (Cancelled)

Docket No: AdVec10IA-C1

Serial No: 09/978,464

10. (previously presented) A recombinant adenovirus vector system comprising:

- (a) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are incapable to encode an infectious, replicable or packageable adenovirus, said first nucleic acid sequence comprising a head-to-head ITR junction and at least one site-specific recombinase recognition target site which is recognized by a site-specific recombinase; and,
- (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are incapable to encode an infectious, replicable or packageable adenovirus, said second nucleic acid sequence comprising a head-to-head ITR junction and a site-specific recombinase recognition target site sufficiently identical with said recombinase recognition target site in said first nucleic acid as to be recognized by the same site-specific recombinase which recognizes said site-specific recombinase recognition target site in said first nucleic acid;

wherein said first and said second nucleic acid sequences, in combination and following site-specific intermolecular recombination, result in production of an infectious adenovirus, and wherein a site-specific recombinase which recognizes said site-specific recombinase recognition target sites either (i) is expressed by a cell into which said first and said second nucleic acids are introduced, (ii) is operatively encoded by said first nucleic acid, said second nucleic acid or both, or (iii) is provided in trans through expression from a third nucleic acid, or (iv) is provided in trans as an active protein.

11. (cancelled)

12. (original) The recombinant adenovirus vector system of claim 10 wherein said cell further expresses adenoviral E1.

Docket No: AdVec10IA-C1

Serial No: 09/978,464

1 13. (previously presented) The recombinant adenovirus vector system of claim 10 wherein
2 said first nucleic acid sequence and said second nucleic acid sequence are cotransfected
3 into said cell to produce an infectious virus vector comprising a left end, a polycloning
4 site or a foreign DNA or an expression cassette from said second nucleic acid sequence,
5 joined to a remaining portion of the adenoviral sequences from said first nucleic acid
6 sequence.

1 14. (cancelled)

1 15. (cancelled)

1 16. (previously presented) A kit for construction of recombinant adenovirus vectors
2 comprising:

3 (A) a first nucleic acid sequence encoding adenovirus sequences which, in the absence
4 of intermolecular recombination, are incapable to encode an infectious, replicable
5 or packageable adenovirus, said first nucleic acid sequence comprising a head-to-
6 head ITR junction and at least one site-specific recombinase recognition target site
7 which is recognized by a site-specific recombinase;

8 (B) a second nucleic acid sequence encoding adenovirus sequences which, in the
9 absence of adenoviral replication factors provided in trans or intermolecular
10 recombination with said first nucleic acid sequence, are incapable to encode an
11 infectious, replicable or packageable adenovirus, said second nucleic acid
12 sequence comprising a head-to-head ITR junction and a site-specific recombinase
13 recognition target site sufficiently identical with said recombinase recognition
14 target site in said first nucleic acid as to be recognized by the same site-specific
15 recombinase which recognizes said site-specific recombinase recognition target
16 site in said first nucleic acid; and

Docket No: AdVec10IA-C1

Serial No: 09/978,464

17 (C) a cell wherein, when said component (a) and said component (b) are cotransfected
18 and recombined through the action of a recombinase which recognizes said
19 recombinase recognition sites, an infectious recombinant adenovirus vector is
20 produced.

1 17 (cancelled)

1 18 (cancelled)